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(71) Applicant *(for all designated States except US)*: **INBIONET CORPORATION** [KR/KR]; 461-6 Jonmin-dong, Yusong-ku, Taejon 305-390 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LEE, Daesang** [KR/KR]; 202, 458-8 Jonmin-dong, Yusong-ku, Taejon 305-390 (KR). **LEE, Mi-Kyoung** [KR/KR]; 306, Wunha Mansion, Misu-dong, Tongyeong 650-080 (KR). **KANG, Key-Jung** [KR/KR]; 415-1 Busa-dong, Jung-ku, Taejon 301-030 (KR). **SHIN, Chul-Soo** [KR/KR]; 113-1101, Samsung-Pureun Apt., Jonmin-dong, Yusong-ku, Taejon 305-390 (KR). **YUN, Jeong-Hwan** [KR/KR]; 105-1302, Dungji Apt., Dunsan 2-dong, Seo-ku, Taejon 302-122 (KR). **YUM, Do-Young** [KR/KR]; 405-1004, Expo Apt., Jonmin-dong, Yusong-ku, Taejon 305-390 (KR). **LEE, Jung-Ki** [KR/KR]; 407-504, Expo Apt., Jonmin-dong, Yusong-ku, Taejon 305-390 (KR). **PARK, Kee-Don** [KR/KR]; 109-1706, Cheonggu-Narae Apt., Jonmin-dong, Yusong-ku, Taejon 305-503 (KR). **CHOI, Ho-Joon** [KR/KR]; 103-406, Sejong Apt., Jonmin-dong, Yusong-ku, Taejon 305-390 (KR). **KOO, Bon-Tag** [KR/KR]; 5-401, Sindonga Apt., Yongjeon-dong, Dong-ku, Taejon 300-200 (KR).

(74) Agent: **KIM, Won-Joon**; 305, Soohyup Bldg, 917, Dunsan-dong, Seo-ku, Taejon 302-828 (KR).

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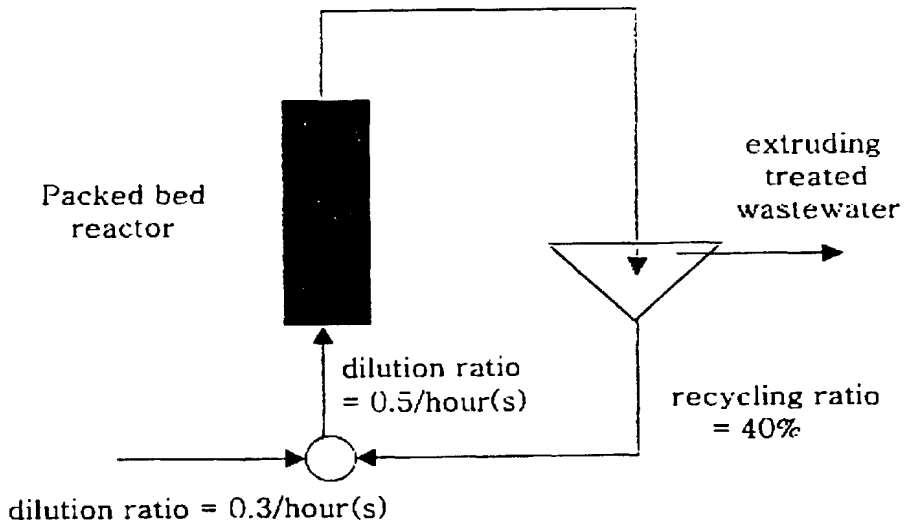
(54) Title: NOVEL STRAIN FOR DECOMPOSING TMAH, AND METHOD OF WASTEWATER TREATMENT USING THE SAME



(57) Abstract: The present invention describes a wastewater treatment method by a microorganism decomposing Tetramethyl Ammonium Hydroxide (TMAH) which, utilized in etching the surface of silicone chip in semiconductor manufacturing process, is toxic and hard to decomposed. The present invention provides novel microorganisms capable of decomposing TMAH. Also, the present invention provides a treatment methdo for wasterwater containing TMAH, using the microorganisms. The present invention is useful in industrial field as an environmental friendly wastewaster treatment method by decomposing over 90 % of TMAH, one of environmental contamimation materials in the wastewater of semiconductor factory.

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NOVEL STRAIN FOR DECOMPOSING TMAH, AND METHOD OF WASTEWATER TREATMENT USING THE SAME

TECHNICAL FIELD

The present invention relates to novel microorganism strains decomposing tetramethyl ammonium hydroxide; TMAH) and wastewater treatment method by applying the above strains.

More particularly, the present invention relates to novel strains decomposing tetramethyl ammonium hydroxide which is utilized for etching the surface of silicon chip while manufacturing semiconductors and is toxic and hard to be degraded. In addition, the present invention relates to wastewater treatment method which comprises applying the above strains into TMAH - containing wastewater and then performing batch culture or various kinds of continuous culture for purging the wastewater.

BACKGROUND ART

Recently, internet and information technology (IT) industries have been developed and the

semiconductor demand also increased explosively. However, the development of the semiconductor industry induces the use of chemical substance gradually. Precisely, tetramethyl ammonium hydroxide (TMAH) which is utilized for etching the surface of silicone chip while manufacturing semiconductors has been exhausted in increased amounts.

TMAH is very toxic (toxicity LC_{50} = 460 ppm) and cannot be biodegraded easily. Hence, TMAH makes biological oxygen demand (BOD) fluctuate extremely in the wastewater treatment facilities of semiconductor manufacturing plant. Therefore, the removal of TMAH from industrial wastewater has become a very important issue in order to protect environment.

Presently, some treatment method has been disclosed for eliminating TMAH from wastewater. In detail, concentration method that comprises concentrating TMAH from the wastewater by using ion exchange resin column, ultra filtration (UF) or reverse osmosis (RO), and disposing TMAH, has been reported. In addition, super-critical treatment method that comprises burning the concentrated TMAH has been demonstrated. Unfortunately, such traditional treatment methods require complicated processes and provokes secondary contamination problems accompanied the said concentration or combustion.

Therefore, more economical and efficient processes for treating TMAH should be developed in order to satisfy the increasing need of environment protection.

To overcome the foregoing and other disadvantages, we, the inventors of the present invention, have developed biological treatment processes which can decompose and eliminate TMAH efficiently even in mild conditions. First, novel microorganism strains which were insensitive to TMAH and utilized TMAH as a sole carbon and energy source have been separated. In addition, we have confirmed that the above strains could reduce the biological oxygen demand (BOD) remarkably when the strains is applied to wastewater containing TMAH.

DISCLOSURE OF INVENTION

The object of the present invention is to provide novel microorganism strains that can degrade tetramethyl ammonium hydroxide (TMAH).

Further object of the present invention is to provide biological treatment methods for TMAH containing wastewater by applying the said strains.

The present invention provides novel microorganism strains capable of decomposing tetramethyl ammonium hydroxide (TMAH), which is often utilized in etching the surface of silicone chip and, is toxic and hard to be degraded. In addition, the present invention provides TMAH containing wastewater treatment methods using the above strains.

The microorganism strains of the present invention is separated and identified by the process comprising steps as follows.

Wastewater sample is obtained from the domestic factory manufacturing semiconductors and is inoculated into the nutrient culture medium for enriching the microorganism of said wastewater primarily. Then, the strains grown above are again cultivated on the Nutrient culture medium containing TMAH. 7 strains out of the above are separated according to the cell survival, namely insensibility to TMAH. In particular, 7 TMAH insensitive strains are first separated and then designated with IBN-H1 ~ IBN-H7 respectively. Furthermore, three strains, IBN-H1, IBN-H4, IBN-H7 superior in decomposing TMAH, are selected among the above strains.

The 3 adopted strains, IBN-H1, IBN-H4, IBN-H7,

are examined by BIOLOG system, MIDI, partial nucleotide sequence determination of 16s RNA and so on. As a result, the above strains are identified to be novel strains corresponding to *Kluyveromyces delphensis*, *Bacillus cereus* and *Acinetobacter sp.* respectively. The strains obtained above are named with *Kluyveromyces delphensis* IBN-H1, *Bacillus cereus* IBN-H4 and *Acinetobacter sp.* IBN-H7 respectively and have been deposited in the Korean Collection for Type Cultures (KCTC) of the Korean Research Institute of Bioscience and Biotechnology (KRIBB), an international deposit organization, #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea on July 18, 2000, and identified as KCTC accession numbers, KCTC 0834 BP, KCTC 0835 BP and KCTC 0836 BP.

It is confirmed that all the strains selected above can grow up to the OD value of 0.2 in the minimal culture medium containing TMAH as a sole carbon source and essential mineral components. In addition, the strains can also grow in both 25°C and 30°C without variations and can grow well even in normal or weakly acidic condition.

The microorganism strains of the present invention are insensitive to TMAH and uses TMAH as a

sole carbon source for cell growth. Hence, they are exploited in the biological wastewater treatment method for removing TMAH. In this process, one strain or more than one strains selected among the group comprising *Kluyveromyces delphensis*, *Bacillus cereus* and *Acinetobacter sp.* can be utilized in sole or mixed culture state, preferably.

The biological wastewater treatment process for removing TMAH of wastewater, which utilizes the microorganism strain of the present invention, can be performed by using the batch culture process. Preferably, the wastewater treatment process is performed by the fed-batch culture or the continuous culture for the efficiency and the continuation of the above process. In the continuous culture system, the microorganism strain is inoculated into a fermentation vessel containing the Nutrient culture medium and is cultivated by using the batch culture process until the density reaches certain point. For example, the OD value is above 0.15. Then wastewater containing TMAH become passed through at the definite dilution velocity.

In the mean time, whatever culture process is adopted, the above stains can be fixed onto a supporting carrier preferably. The carrier facilitates said processes since it enables the strains to be used

repeatedly and to remove wastewater conveniently. The carrier substance can be selected preferably among alginate, urethane foam and so on, which are conventional material for the microorganism fixation.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the survival density of the strains of the present invention according to the TMAH concentration;

FIG. 2a shows the growth curves of IBN-H1 strain according to cell incubation temperature;

FIG. 2b shows the growth curves of IBN-H4 strain according to incubation temperature;

FIG. 2c shows the growth curves of IBN-H7 strain according to incubation temperature;

FIG. 3a shows the growth curves of IBN-H1 strain according to culture pH;

FIG. 3b shows the growth curves of IBN-H4 strain according to culture pH;

FIG. 3c shows the growth curves of IBN-H7 strain according to culture pH;

FIG. 4 shows the batch-cultured growth curves of the strains of the present invention;

FIG. 5 shows the continuous-cultured growth curves of the IBN-H1 strain of the present invention;

FIG. 6 shows the diagram of the recycling equipment for continuous wastewater treatment of the present invention;

FIG. 7 shows the growth curves of the strains according to wastewater treatment by using the recycling equipment disclosed in **FIG. 6**;

FIG. 8 shows the diagram of the wastewater treatment equipment that exploits the packed bed reactor of the present invention;

FIG. 9a shows the schematic diagram of the pilot system that represents the biological treatment process of TMAH by applying the microorganism strains of the present invention;

FIG. 9b shows the detailed diagram of pilot system that represents the biological treatment process of TMAH by applying the strains of the present invention; and,

FIG. 10 shows the cell concentration as a function of the inlet TMAH concentration in the pilot system.

BEST MODE FOR CARRYING OUT THE INVENTION

Practically and presently preferred embodiments of the present invention are illustrative as shown below.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the scope of the present invention.

Preferred Embodiment 1: Separation of microorganism strains insensitive to TMAH

(1) Proliferation of cell strains existed in wastewater of semiconductor factory

Wastewater samples, such as influent, mixed water

and return sludge were obtained from the wastewater treatment process of domestic semiconductor manufacturing companies.

The samples prepared above were diluted successively and smeared onto the Nutrient agar plate including sufficient carbon sources (beef extract 0.3% (DIFCO), peptone 0.5% (DIFCO), agar 1.5% (DIFCO)). Then the plate was cultivated with incubator at 30°C for 18 hours.

(2) Separation of TMAH insensitive strains

In order to separate the strains of the present invention, the insensibility to TMAH was examined in all the strains obtained from the above proliferation stage.

The Nutrient culture medium including sufficient carbon sources (beef extract 0.3% (DIFCO); peptone 0.5% (DIFCO)) was prepared, in which TMAH concentration was adjusted to 0.25%, 0.5%, 1.0% and 2.0% respectively. Then the strains proliferated above were inoculated and were cultivated with incubator at 30°C by shaking at 250 rpm velocity.

By the process described above, the 7 strains which proliferated and were distinguished with naked eyes were selected from the culture medium. The 7

strains were named with IBN-H1 ~ IBN-H7 respectively. Then the final culture medium solutions obtained above were suspended with the successive dilution method and were smeared onto the above Nutrient agar plate. In addition, the total cell number was represented as colony forming unit (CFU) appearing from 1 ml of culture medium (see **Fig. 1**).

As depicted in **Fig. 1**, all the 7 strains selected from the above process have seldom grown in the medium condition containing more than 1.7% of TMAH. In particular, the IBN-H3, IBN-H4, IBN-H5 and IBN-H7 strains can proliferate actively at the concentration less than the above.

Preferred Embodiment 2: Examination of TMAH decomposition ratio by the TMAH insensitive strains separated above

The TMAH decomposition ratio by the TMAH insensitive strains selected in Preferred Embodiment 1 was measured as follows.

The minimal culture medium including essential mineral components for cell growth ($(NH_4)_2SO_4$ 3g/L, trace element (H_3BO_3 0.3g, $CoSO_4 \cdot 7H_2O$ 0.2g, $ZnSO_4 \cdot H_2O$ 0.1 g, $MnCl_2 \cdot H_2O$ 0.03 g, $NaMoO_4 \cdot H_2O$ 0.03 g, $NiCl_2 \cdot H_2O$ 0.02 g, $CuSO_4 \cdot H_2O$ 0.01 g, $FeSO_4 \cdot H_2O$ 20 g, $CaCl_4 \cdot$

H_2O 10 g/L) 2 ml/L, $MgSO_4 \cdot 7H_2O$ (40%) 2 ml/L, 1 M of phosphate salt buffer solution (pH 7)) was mixed with 1% TMAH as a carbon source. Then the strains of the present invention were cultivated with the above culture medium by shaking at 30°C for 72 hours.

The BOD of the final culture medium was measured by performing triple tests. As a result, the removal ratio of TMAH was confirmed to be more than 90% in the IBN-H1, IBN-H4, IBN-H6 and IBN-H7 strains (see **Table 1**).

<Table 1>

Removal ratio of TMAH in the strains of the present invention

TAG	TMAH conc. before dilution	TMAH conc. after 10^{-2} dilution	expected BOD	BOD				removal ratio
				sample	DO_0	DO_5	BOD_5	
IBN-H1	**1%**	**100 ppm**	**180→150~50**	**10**	**9.1**	**8.75**	**10.5**	**94.2%**
IBN-H2	1%	100 ppm	180→150~50	10	9.15	6.55	78	56.7%
IBN-H3	1%	100 ppm	180→150~50	10	9.2	6.45	82.5	54.2%
IBN-H4	**1%**	**100 ppm**	**180→150~50**	**10**	**9.2**	**9.05**	**4.5**	**97.5%**
IBN-H5	1%	100 ppm	180→150~50	10	9.15	6.2	88.5	50.8%
IBN-H6	1%	100 ppm	180→150~50	10	9.15	8.65	15	91.7%
IBN-H7	**1%**	**100 ppm**	**180→150~50**	**10**	**9.2**	**9.05**	**4.5**	**97.5%**

Preferred Embodiment 3: Identification of the microorganism strains with excellent activity in decomposing TMAH

According to the processes of the Preferred Embodiments 1 and 2, 3 kinds of strains, IBN-H1, IBN-H4 and IBN-H7, showing excellent activities in decomposing TMAH were adopted. Then BIOLOG experiment, fatty acid analysis, MIDI, 16S rRNA partial nucleotide sequencing and so on were performed in order to identify the above strains.

Above all, the morphological and biochemical characteristics in the above strains of the present invention were investigated. In detail, all the 3 strains of the present invention retained catalase activity. And in Gram dyeing reaction for identifying bacteria, the IBN-H4 strain was confirmed as gram positive and the IBN-H7 strain was confirmed as gram negative (See **Table 2**). On the other hand, the IBN-H1 strain was not applied for the Gram dyeing reaction since it was an yeast.

<Table 2>

Morphological and biochemical properties in the strains of the present invention

	IBN-H1	IBN-H4	IBN-H7
Mobility	no	no	no
Gram dyeing	-	positive	negative
Catalase activity	positive	positive	positive
Others	aerobic	aerobic	aerobic

In order to accomplish the BIOLOG analysis for identifying the IBN-H1 strain, the cell suspension of the above strain was inoculated onto 96-well microplate that carbon source of dried state was added into and was cultivated. Then the color variation and turbidity were recognized and analyzed by comparing the results with database which consisted in the results of more than 1,400 species of standard strains (aerobic strain, anaerobic strain and yeast strains). Consequently, the IBN-H1 strain was identified to belong to *Kluyveromyces delphensis* (See **Table 3**).

<Table 3>

Relationship of similar strains with IBN-H1 strain of the present invention

Similar strain	SIM	DIST	AVG	MAX
Kluyveromyces delphensis	0.623	5.288	0.569	1.094

Candida glabrata	0.026	6.338	0.500	1.487
Pichia pastoris	0.002	7.239	0.750	2.338
Pichia amenthionina var pachy	0.001	7.373	1.150	6.524
Candida fluctus	0.000	7.635	0.313	0.606
Candida zeylanoides	0.000	7.997	0.625	3.537
Phodotorula hylophila	0.000	8.119	2.399	9.845

The fatty acid contents of the IBN-H4 and IBN-H7 strains were analyzed as follows. In the IBN-H4 strain, fatty acids with carbon number 15 were the major component and in IBN-H7 strain fatty acids with carbon number 18 were the major component resultantly (See **Table 4**). As depicted in **Table 2**, in the fatty acids of the IBN-H4 strain having carbon number 15, 23.71% was iso type which had no double bond and 8.49% was anteiso type.

<Table 4>

Analysis of cellular fatty acids within the strains of the present invention

	IBN-H4	**IBN-H7**
C12:0		8.42
C12:0 2OH		2.12
C12:0 3OH		6.66

C12:0 iso	1.08	
C13:0 iso	7.48	
C13:0 anteiso	2.24	
C14:0 iso	6.26	
C14:0	2.28	1.82
C15:0 iso	23.71	
C15:0 anteiso	8.49	
C16:1 iso I/C 14:0 3OH	3.37	
C16:0 iso	9.40	
C16:1 w11c		
C15:0 iso 2OH/C 16:1 w7c	8.84	30.62
C16:0	6.72	14.31
C15:0 iso 3OH		
Iso C17:1 w10c	1.76	
Iso C17:1 w5c	3.87	
C17:0 iso		
C17:0 anteiso A	1.61	
C17:0 iso	9.02	
C17:0 anteiso	3.03	
C18:1 w9c		32.94
C18:1 w7c	3.03	1.69

In order to examine the sequence homology, 16s RNA partial nucleotide sequence analysis was performed by using the strains of the present invention respectively (See Sequence List; sequence number 1 and sequence number 2). Consequently, the IBN-H4 strain was shown to have 100% homology with *Bacillus cereus* IAM 12605T (See **Table 5**) and the IBN-H7 strain had 94.95% homology with *Acinetobacter calcoaceticus* ATCC

23055T (See **Table 6**). Therefore, the IBN-H4 and IBN-H7 strain was confirmed to belong to respective *Bacillus cereus* and *Acinetobacter sp.*

<Table 5>

The analysis data of 16S rRNA homology in the IBN-H4 strain of the present invention

Strains	Homology (%)	No. of non-identical nucleotides / No. of total nucleotides
Bacillus cereus IAM 12605T	100.00	0/584
Bacillus thuringiensis IAM 12077T	99.49	3/584
Bacillus mycoides DSM 2048T	99.30	4/571
Bacillus weihenstephanensis DSM 11821T	99.13	5/573
Bacillus pseudomycoides DSM 12442T	98.63	8/584
Bacillus *atrophaeus* NCIB 12899T	92.55	41/550
Bacillus megaterium IAM 13418T	92.45	44/583
Bacillus cohnii DSM 6307T	92.44	44/582
Bacillus halmapalus DSM 8723T	92.41	44/580
Bacillus subtilis ATCC 6051T	92.08	46/581
Alicyclobacillus acidoterrestris DSM 3922T	91.83	47/575
Bacillus amyloliquefaciens ATCC 23350T	91.81	47/574
Bacillus licheniformis ATCC 14580T	91.57	49/581
Bacillus sporothermodurans DSM 10599T	91.44	50/584
Bacillus simplex DSM 1321T	91.21	51/580
Bacillus azotoformans ATCC 29788T	91.12	49/552
Bacillus psychrosaccharolyticus ATCC 23296T	91.09	50/561
Bacillus oleronius DSM 9356T	91.08	52/583

Bacillus pumilus ATCC 7061T	90.01	51/567
Bacillus circulans ATCC 4513T	90.97	50/554
Bacillus lentus IAM 12466T	90.86	53/580
Bacillus benzoevorans ATCC 49005T	90.81	53/577
Bacillus fastidiosus DSM 91T	90.64	54/577
Bacillus firmus IAM 12464T	90.50	55/579
Bacillus badius ATCC 14574T	90.34	56/580
Bacillus marinus DSM 1297T	90.19	57/581

<Table 6>

The analysis data of 16S rRNA homology in the IBN-H7 strain of the present invention

Strains	**Homology (%)**	**No. of non-identical nucleotides / No. of total nucleotides**
Acinetobacter calcoaceticus ATCC 23055T	94.95	26/515
Acinetobacter haemolyticus DSM 6962T	94.17	30/515
Acinetobacter baumannii DSM 30007T	93.98	31/515
Acinetobacter lwoffii DSM 2403T	92.82	37/515
Acinetobacter radioresistens DSM 6976T	92.77	37/512
Acinetobacter johnsonii DSM 6963T	92.23	40/515
Acinetobacter junii DSM 6964T	91.47	44/516

Preferred Embodiment 4: TMAH availability in the identified strains of the present invention

The IBN-H1, IBN-H4 and IBN-H7 strains of the

present invention identified above were examined in order to detect whether TMAH could be utilized as a sole carbon source.

The minimal culture medium including essential mineral components for cell growth (reference: Preferred Embodiment 2) was utilized and mixed with 0.5% TMAH as a carbon source. Then the above strains were inoculated independently and cultivated at 30°C for 72 hours by shaking. This culture medium was diluted successively and the diluted cell suspension was smeared onto the culture plate having the same composition of the above culture medium and incubated.

The 3 strains of the present invention were observed to form colonies onto the culture plate respectively, which confirmed that the above strains could grow by using TMAH as a sole carbon source and as an energy source.

In order to investigate the difference of the strains, the availability of TMAH was examined in the strains of the present invention and other strains related with the above stains.

In detail, *Kluyveromyces dephensis* and *Saccharomyces cerevisiae* were utilized instead of the IBN-H1 strain; *Bacillus cereus*, *Bacillus subtilis* and *Bacillus brevis* instead of the IBN-H4 strain; and

Acinetobacter calcoaceticus and *Acinetobacter genospecies* instead of the IBN-H7 strain respectively in order to disclose whether TMAH could be adopted as a carbon source or not.

Consequently, all the comparative strains having relationship with the strains of the present invention did not form colonies. Hence the comparative strains were confirmed not to utilize TMAH as a carbon source (See **Table 7**).

Therefore, the strains of the present invention were identified again to have different physiological and biochemical properties from those of conventional strains, although the strains of IBN-H1, IBN-H4 and IBN-H7 in the present invention belonged to *Kluyveromyces dephensis*, *Bacillus cereus* and *Acinetobacter sp.* respectively.

<Table 7>

Examination for the usage of TMAH as a carbon source in the selected strains of the present invention and comparative strains related with the above strains

The stains of the present invention (comparative strains related)	**Colony forming (yes/no)**
***Kluyveromyces delphensis* IBN-H1**	**yes**
(*Kluyveromyces delphensis*)	no
(*Saccharomyces cerevisiae*)	no

***Bacillus cereus* IBN-H4**	**yes**
(*Bacillus cereus*)	no
(*Bacillus subtilis*)	no
(*Bacillus brevis*)	no
***Acinetobacter sp.* IBN-H7**	**yes**
(*Acintobacter calcoaceticus*)	no
(*Acintobacter genospecies*)	no

Preferred Embodiment 5: TMAH insensibility of the identified strains

The IBN-H1, IBN-H4 and IBN-H7 strains of the present invention identified above were examined in order to detect the TMAH insensibility.

The cell strains of the present invention and the related strains were inoculated into the Nutrient culture medium (Bacto beef extract 3g/L, Bacto peptone 5g/L) with 3 ml volume respectively and were cultivated at 30°C for 18 hours by shaking. Then the culture medium of each strain was allotted to measure the absorbance (OD) at 600 nm, which would adjust the density and number of the cell strains and could facilitate the insensibility experiments. The above samples were centrifuged at 12,000 rpm for 5 minutes and were washed 3 times with saline for eliminating the remaining medium. Then the sample were resuspended by

adding 1 ml of 2% TMAH and maintained at 30°C for an hour. The cell strains were again centrifuged in order to remove the cell supernatant and washed 3 times with saline so as to remove the remaining TMAH. Then the cell samples obtained were diluted with 200 μl of physiological saline successively and were resuspended. The cell suspensions were smeared onto the agar culture plate containing Nutrient medium components and were incubated at 30°C for 18 hours. And then the number of colonies on the above agar plate was calculated in each strain.

For controlled samples, physiological saline was utilized instead of 2% TMAH in each cell strains. In addition, the number of colonies formed in each experimental sample and comparative sample was adjusted reciprocally with that in the controlled sample, which had no TMAH for measuring exactly.

In order to compare the insensibility to TMAH, both the strains of the present invention and the strains related with the above strains were investigated for the detection.

Precisely, as a comparative strain related with the IBN-H1 strain of the present invention *Kluyveromyces dephensis* and *Saccharomyces cerevisiae* were applied; *Bacillus cereus*, *Bacillus subtilis* and

Bacillus brevis as a strain related with the IBN-H4 strain; and *Acinetobacter calcoaceticus* and *Acinetobacter genospecies* as a strain related with the IBN-H7 strain respectively.

Consequently, all the comparative strains having relationship with the strains of the present invention formed even smaller number of colonies than the strains of the present invention. Hence the comparative strains were confirmed to be much less insensitive to TMAH (See **Table 8**).

Therefore, the strains of the present invention, IBN-H1, IBN-H4 and IBN-H7, were also identified to be new strains having different physiochemical properties from those of conventional strains although the above strains belonged to *Kluyveromyces dephensis*, *Bacillus cereus* and *Acinetobacter sp.* respectively.

As demonstrated above, the above strains of the present invention, IBN-H1, IBN-H4 and IBN-H7, are confirmed as new strains which have not been reported. The strains obtained above are named with *Kluyveromyces delphensis* IBN-H1, *Bacillus cereus* IBN-H4 and *Acinetobacter sp.* IBN-H7 respectively and have been deposited with the Korean Collection for Type Cultures (KCTC) of the Korean Research Institute of Bioscience and Biotechnology (KRIBB), an international

deposit organization, #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea on July 18, 2000, and identified as KCTC accession numbers, KCTC 0834 BP, KCTC 0835 BP and KCTC 0836 BP.

<Table 8>

Comparison of the insensibility to TMAH in the selected strains of the present invention and the related strains

The stains of the present invention (comparative strains related)	**Insensibility to TMAH**
***Kluyveromyces delphensis* IBN-H1**	**100%**
(*Kluyveromyces delphensis*)	< 10 ± 5%
(*Saccharomyces cerevisiae*)	< 10 ± 5%
***Bacillus cereus* IBN-H4**	**100%**
(*Bacillus cereus*)	< 10 ± 5%
(*Bacillus subtilis*)	< 10 ± 5%
(*Bacillus brevis*)	< 10 ± 5%
***Acinetobacter sp.* IBN-H7**	**100%**
(*Acintobacter calcoaceticus*)	< 10 ± 5%
(*Acintobacter genospecies*)	< 10 ± 5%

Preferred Embodiment 6: Characteristics in cell growth of the identified strains

(1) Characteristics of cell growth according to incubation temperature

In order to detect the optimal temperature for cultivating the separated strains of the present invention, the above strains were suspended by using the same culture medium with that used in Preferred Embodiment 2 and 200 μl of the culture medium was added into 96-well microplate. Then the cell in the microplate was cultured at 25°C and 30°C for 72 hours by shaking at 250 rpm. The OD values of the cell strains were calculated at 600 nm by using microplate reader (MR 5000, Dynatech, USA) (see **Fig. 2a**, **Fig. 2b** and **Fig. 2c**).

As described in **Fig. 2a**, **Fig. 2b** and **Fig. 2c**, the cell strains proliferated more actively at 30°C than at 25°C during the whole culturing process. In addition, the cell numbers (OD value) were maintained almost constant through culturing period at each temperature, particularly about 0.2, 0.05 in the IBN-H1 strain, about 0.15, 0.15 in the IBN-H4 strain and about 0.25, 0.15 in the IBN-H7 strain.

(2) Characteristics of cell growth according to pH

In order to examine the optimal pH for culturing the separated strains of the present invention, the above strains was suspended by using the same culture medium with that used in Preferred Embodiment 3 and 200 μl of the culture medium was added into 96-well microplate. Then the cell in the microplate was cultured at pH 5, pH 7 and pH 9, at 30°C for 72 hours by shaking at 250 rpm. The OD values of the strains were calculated at 600 nm by using microplate reader (MR 5000, Dynatech, USA) (see **Fig. 3a**, **Fig. 3b** and **Fig. 3c**).

As described in **Fig. 3a**, **Fig. 3b** and **Fig. 3c**, in the IBN-H1 strain, the OD value in PH 5 to PH 7 range was about 0.2; in the IBN-H4 strain, the OD value at pH 7 ranges was about 0.15; and in the IBN-H7 strain, OD value at pH 7 range was 0.2 approximately. Hence, the above 3 strains were known to grow well in both the normal and weakly acidic condition.

(3) Characteristics of cell growth in batch culture by using culture medium containing 1% TMAH

In order to examine the growth pattern of the

above strains while performing batch culture by using TMAH - containing culture medium, the above strains IBN-H1, IBN-H4 and IBN-H7 were cultivated with the same culture medium of Preferred Embodiment 2 at 30°C for 72 hours by shaking at 250 rpm. The OD values of the above strains were calculated at 600 nm in every hour interval by using microplate reader (MR 5000, Dynatech, USA) and then the growth curves of the each cell strain were drawn (see **Fig. 4**).

Consequently as described in **Fig. 4**, the IBN-H1 and the IBN-H4 strains reached the maximum value of the cell growth (OD value: 2.24 and 1.80) after about 22-hour culture and the IBN-H7 strains increased the growth rate slowly until 100 hours and reached the maximum value (OD value: 2.36).

Preferred Embodiment 7: Biological TMAH treatment process by using the strains of the present invention

The possibility of the above strains for biological treatment was investigated indirectly by applying the water treatment model for the TMAH - containing wastewater and then by monitoring the cell number of the strains in culture medium.

The volume of the fermentator was 5 L, if not mentioned especially, and the initial amount of the

culture medium was 3 L and the incubation temperature was 30°C.

(1) Enrichment of the strains for applying to the treatment process

In order to make practical application for the TMAH - containing wastewater treatment, the cell strains obtained in the present invention were cultivated with high density respectively.

The above strains were inoculated into the fermentation vessel with 5 L volume including 3L of Nutrient culture medium with glucose (glucose 10 g/L, yeast extract 10 g/L, $MgSO_4 \cdot 7H_2O$ 1.2g/L, KH_2PO_4 13.3 g/L, $(NH_4)_2 \cdot HPO_4$ 4 g/L, pH 7.0 (HCl)) independently and were cultivated at 30°C. In every 24-hour interval, high Nutrient culture medium (glucose 274 g/L, yeast extract 211 g/L, $MgSO_4 \cdot 7H_2O$ 1 g/L, $(NH_4)_2 \cdot HPO_4$ 1.5 g/L, pH 7.0 (HCl)) was added again by 100 ml volume. According to the process, the fed-batch culture was accomplished.

Consequently, all the 3 strains of the present invention sustained the high density which was more than 40 in the OD value. Hence, it was confirmed that the above strains could multiply so as to be applied for industrial uses.

(2) Batch culture treatment

The above strains, IBN-H1, IBN-H4 and IBN-H7 were inoculated into the fermentation vessel with 5 L volume including 3L of Nutrient culture medium (yeast extract 1 g/L, $MgSO_4 \cdot 7H_2O$ 0.8 g/L, KH_2PO_4 1 g/L, $(NH_4)_2 \cdot HPO_4$ 1 g/L, pH 7.0 (HCl)) with 1.0% TMAH independently and were cultivated at 30°C for 70 hours in batch pattern. As a result, the OD value at 660 nm reached 2.2, 1.8 and 2.5 respectively.

Consequently, all the 3 strains of the present invention sustained the TMAH insensibility even in large scale and can utilize TMAH as a sole carbon source for cell growth. Hence, it was confirmed that the above strains could be exploited to treat wastewater containing TMAH in the batch culture pattern.

(3) Continuous culture treatment

The above strains, IBN-H1, IBN-H4 and IBN-H7 were cultivated respectively by using the same treatment process of the above batch culture. Then at the point that the OD value reached to 2.2, 1.8 and 2.5 respectively, the TMAH - containing culture medium and water were passed through with 0.15 of dilution ratio

per hour for treating continuously.

Consequently, the OD values have decreased from the start point of the continuous culture and were maintained stably after about 60 hours in near 1.1, 0.9 and 1.2 of the OD value respectively (See **Fig. 5**).

Therefore, it was confirmed that all the 3 strains of the present invention sustained considerable cell densities even in the continuous treatment process and could be exploited to treat TMAH - containing wastewater in the continuous pattern.

In Preferred Embodiments described below, the above 3 strains of the present invention were cultivated with high densities and were mixed in the same ratio's and then were applied for performing the experiments.

(4) Recycling continuous culture treatment

As shown in **Fig. 6**, the recycling type continuous culture was performed by the process as follows. Precisely, the culture medium outflowing from the continuous treatment process was passed through cell separation unit which was composed of fibrous strand; and then recovered into the fermentation vessel.

At the initial stage, simple batch culture was

performed. But if the OD value reached 2.0, the culture pattern was changed to the recycling continuous culture. The dilution ratio was 0.3 per hour and the plain medium corresponding to 80% volume of the culture medium was extruding from the fermentator finally. Namely, the reflection rate was adjusted to 0.2.

Consequently, the OD value of the culture medium was maintained in 6 ~ 7 range stably and long when about 50 hours has passed from the initiation point (See **Fig. 7**).

Therefore, it was confirmed that the TMAH - containing wastewater could also be treated efficiently even by using the cell strains of the present invention and the recycling continuous culture process.

(5) Continuous culture treatment by using fluidized - bed reactor

According to the above process of continuous culture treatment, the cell strains were cultivated. If the OD value reached 0.2, conventional polyurethane foam (standard size; 0.5 × 0.5 × 0.5 cm^3) which has been used in fermentator was added with 90% of the void volume for the absorption of the above strains. The resulting support with the cell strains was incubated in the continuous culture pattern.

Consequently, the same cell density can be sustained with that obtained from the simple continuous culture process although the dilution ratio (0.3 per hour) was even higher than that of the above simple continuous culture (0.5 per hour).

(6) Continuous culture treatment by using packed bed reactor

The packed bed reactor (reactor standard = radius 10, height 30 cm; void volume = 70%) which was fulfilled with the polyurethane foam supporting the cell strains of the present invention was exploited. The culture medium and recycling water were passed upward in the dilution ratio 0.3/hour and 0.2/hour respectively through the above packed bed reactor and pressure (namely, dilution ratio 0.5/hour while using the reactor as a standard). 40% (volume ratio) of extruding water from the reactor was recovered and reused with recycling water (See **Fig. 8**).

Consequently, the OD value was maintained at 1.4 stably during 2 months and the biological oxygen demand (BOD) has reduced from 466.5 to 169.7, which corresponded to 74% reduction.

Preferred Embodiment 8: Pilot system experiment of biological TMAH treatment process by using the strains of the present invention

In order to treat wastewater containing TMAH, the pilot system was established in the semiconductor manufacturing factory (Hyundai Electronics Industries Co., Ltd; Chungju). The mixed strains composed of the novel strains of the present invention were applied for performing the biological wastewater treatment.

The cell recycle bioreactor having 200 L of volume capacity and 120 L of working capacity was used and a recycle filtration membrane (ceramic material, 0.1 μm of pore size) was adopted for preventing cells from outflowing (See **Fig. 9a** and **Fig 9b**).

Generally, the TMAH concentration in discharging wastewater from the factory was comparatively low in 3 ~ 4 g/L. Hence, the concentrated TMAH solution was added into the wastewater directly for the convenience of the experiment. In detail, the TMAH concentration of the wastewater was adjusted to reach 5 ~ 15 g/L. By adding the concentrated TMAH solution to the outflowing wastewater in this Preferred Embodiment for the convenience. However, reverse osmosis system (RO) would be exploited in the practical application in order to concentrate TMAH of

wastewater highly.

In addition, since the original wastewater contained excessive amount of TMAH relatively, the culture medium containing proper nitrogen sources (per 15 g TMAH: containing yeast extract 0.1 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.05 g) should be mixed to make Nutrient state required for the cell growth.

At that time, temperature (25 ~ 40°C), air volume (1 vvm), pH (6.5 ~ 7.2) and so on was maintained properly and flow rate of wastewater and retention period were adjusted to 30 L/hour and 4 hour respectively during the treatment, which helped the cell growth environmentally.

According to the reaction period and TMAH concentration of wastewater, the dried cell concentration was changed as demonstrated in **Fig. 10** and the results were summarized in **Table 9**. As shown in **Table 9**, it was confirmed that the TMAH wastewater (5 ~ 15 g/L) having various concentrations was treated successfully (treatment efficiency 94 ~ 99%).

<Table 9>

Pilot experiment of biological TMAH treatment process by using the strains of the present invention.

TMAH conc. of inflowing wastewater	BOD5 (ppm) of inflowing wastewater	BOD5 (ppm) of wastewater treated	Conc. of stable cell strains (g/L)	Treatment efficiency (%)
5	10,000	50~70	-	>99
8	16,000	400~600	24~25	96~97.5
12	24,000	1,000~400	36~37	94~95
15	30,000	400~600	43~44	>98

INDUSTRIAL APPLICABILITY

The present invention relates to novel strains decomposing tetramethyl ammonium hydroxide, which is often utilized in etching the surface of silicone chip while manufacturing semiconductors and is toxic and hard to be degraded. In addition, the present invention relates to a wastewater treatment method which comprises cultivating the above strains by using batch culture processes; applying the culture medium into TMAH containing wastewater; and performing continuous culture processes.

The novel strains of the present invention and the wastewater treatment process can decompose environmental contaminants TMAH over 95% existed in the wastewater of the semiconductor manufacturing facilities. Therefore, the wastewater treatment

method of the present invention can be applied to industries as an environmental friendly wastewater treatment system efficiently.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the scope of the invention as set forth in the appended claims.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 23, line 22.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution
Korean Collection for Type Culture (KCTC)

Address of depositary institution *(including postal code and country)*

Korean Research Institute of Bioscience and Biotechnology (KRIBB)
#52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea

Date of deposit	Accession Number
July 18, 2000	KCTC 0834BP

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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#52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea

Date of deposit	Accession Number
July 18, 2000	KCTC 0835BP

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Address of depositary institution *(including postal code and country)*

Korean Research Institute of Bioscience and Biotechnology (KRIBB)
#52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea

Date of deposit	Accession Number
July 18, 2000	KCTC 0836BP

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E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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What is claimed is:

1. *Kluyveromyces delphensis* IBN-H1 strain (accession number : KCTC 0834 BP) which is insensitive to tetramethyl ammonium hydroxide (TMAH) and uses TMAH as a carbon source for cell growth.

2. *Bacillus cereus* IBN-H4 strain (accession number : KCTC 0835 BP) which is insensitive to TMAH and uses TMAH as a carbon source for cell growth.

3. *Acinetobacter sp.* IBN-H7 strain (accession number : KCTC 0836 BP) which is insensitive to TMAH and uses TMAH as a carbon source for cell growth.

4. A biological wastewater treatment method for removing tetramethyl ammonium hydoxide of wastewater, which utilizes one strain or more than one strains selected among the group comprising *Kluyveromyces delphensis* of Claim 1, *Bacillus cereus* of Claim 2 and *Acinetobacter sp.* Of Claim 3.

5. The biological wastewater treatment method for removing tetramethyl ammonium hydoxide of wastewater according to Claim 4, in which

treatment is performed by batch culture.

6. The biological wastewater treatment method for removing tetramethyl ammonium hydoxide of wastewater according to Claim 4, in which treatment is performed by continuous culture.

7. The biological wastewater treatment method for removing tetramethyl ammonium hydoxide of wastewater according to Claim 6, in which the microorganism strain/strains is/are fixed onto a supporting carrier.

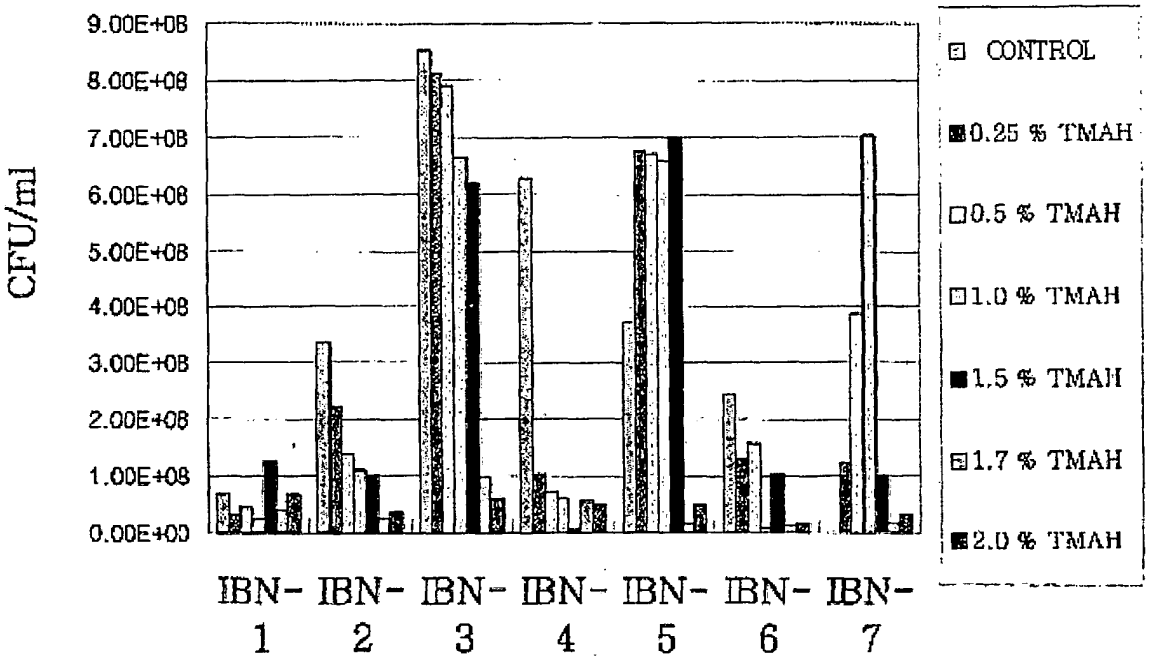
9.00E+08
8.00E+08
7.00E+08
6.00E+08
5.00E+08
4.00E+08
3.00E+08
2.00E+08
1.00E+08
0.00E+00
CFU/ml
IBN-1
IBN-2
IBN-3
IBN-4
IBN-5
IBN-6
IBN-7
CONTROL
0.25 % TMAH
0.5 % TMAH
1.0 % TMAH
1.5 % TMAH
1.7 % TMAH
2.0 % TMAH

Fig. 1

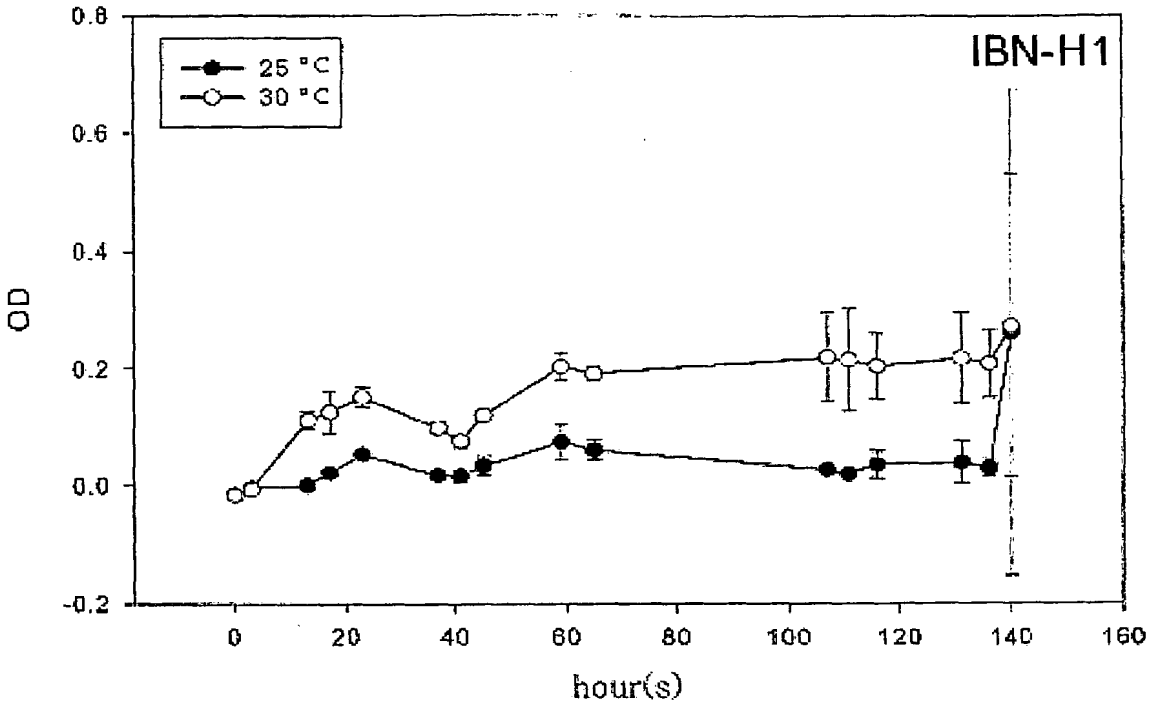
IBN-H1
25 °C
30 °C
OD
0.8
0.6
0.4
0.2
0.0
-0.2
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 2a

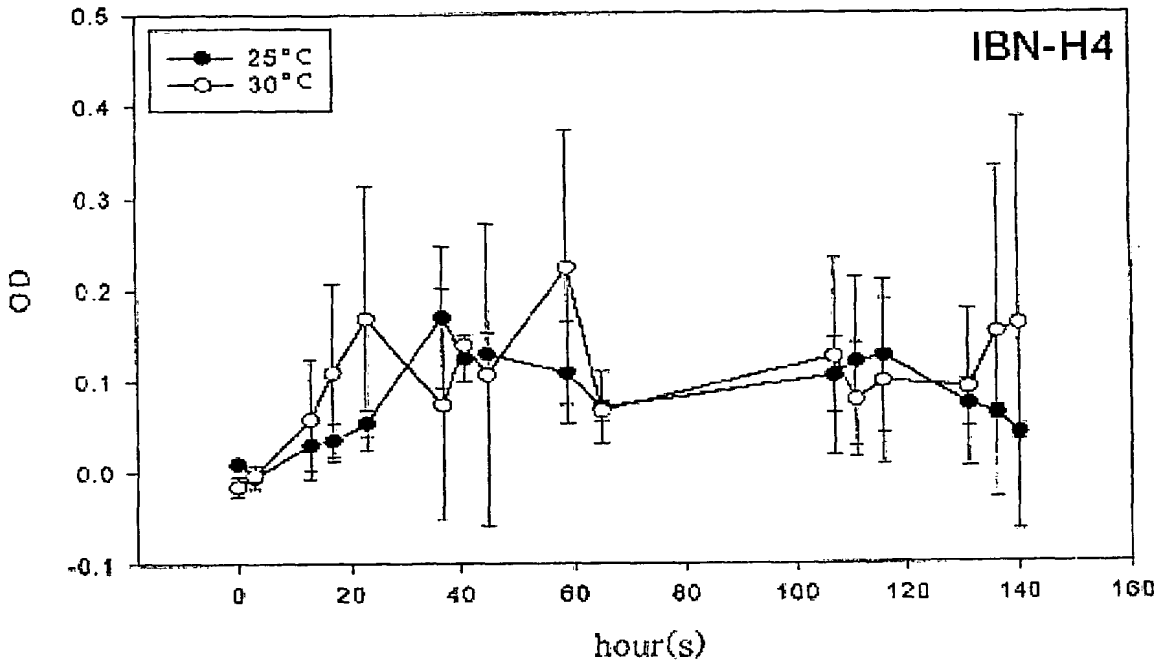
IBN-H4
25°C
30°C
OD
hour(s)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
20
40
60
80
100
120
140
160

Fig. 2b

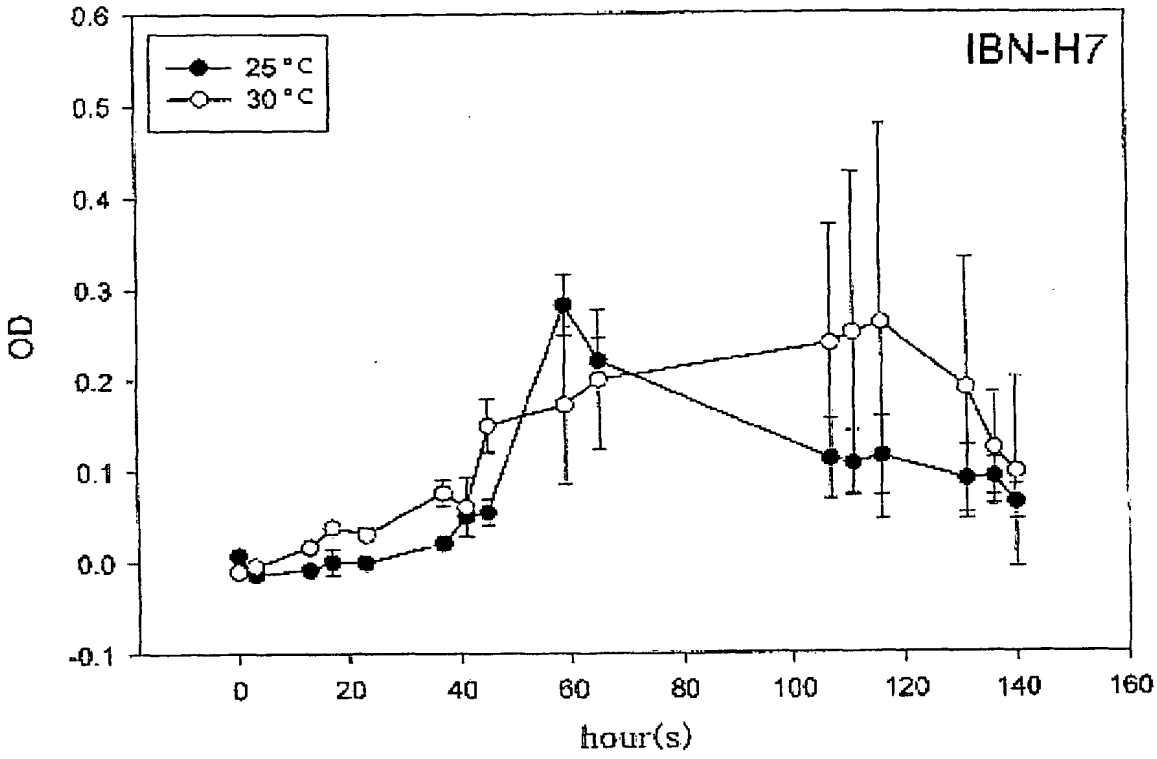
IBN-H7
25 °C
30 °C
OD
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 2c

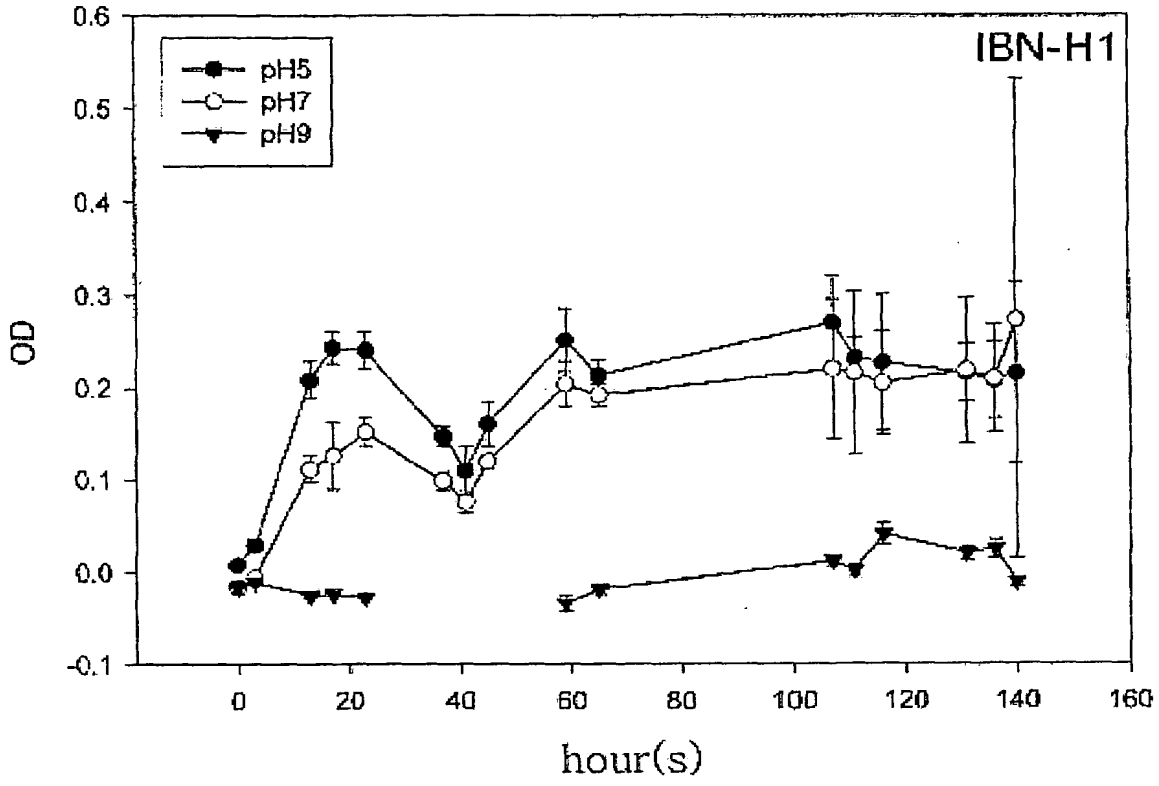
IBN-H1
pH5
pH7
pH9
OD
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 3a

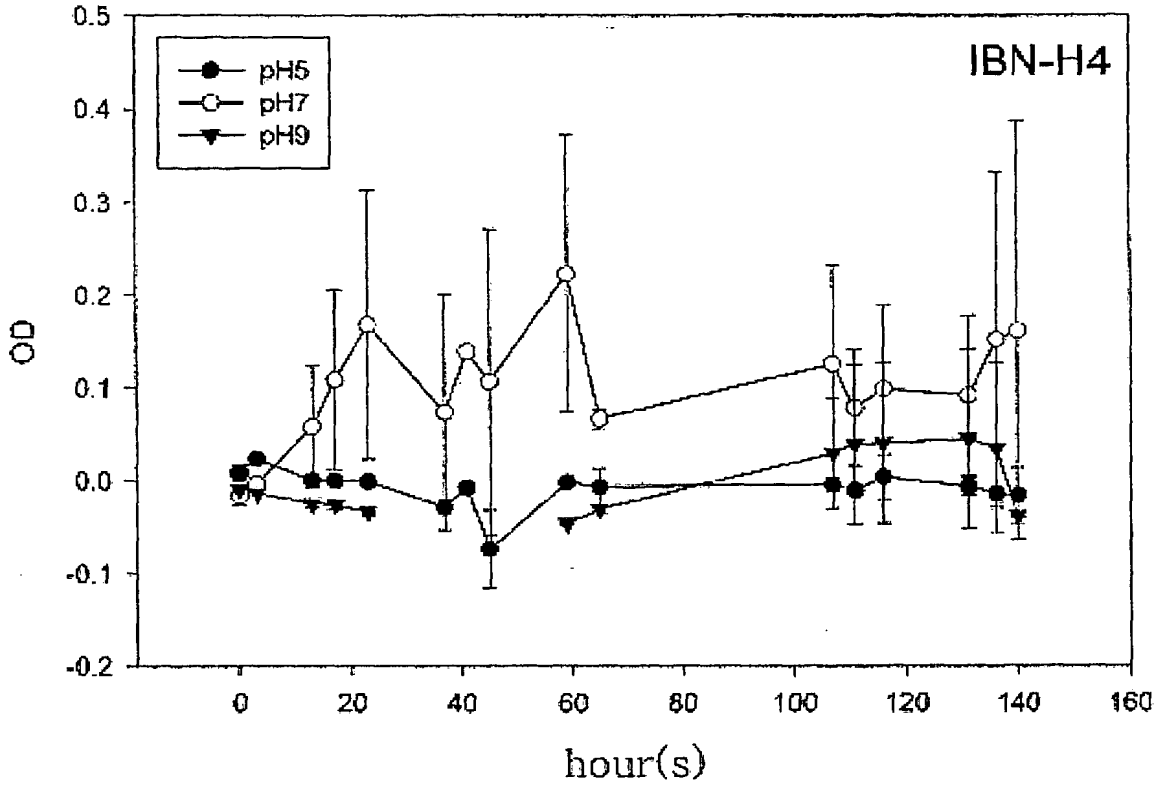
IBN-H4
pH5
pH7
pH9
OD
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 3b

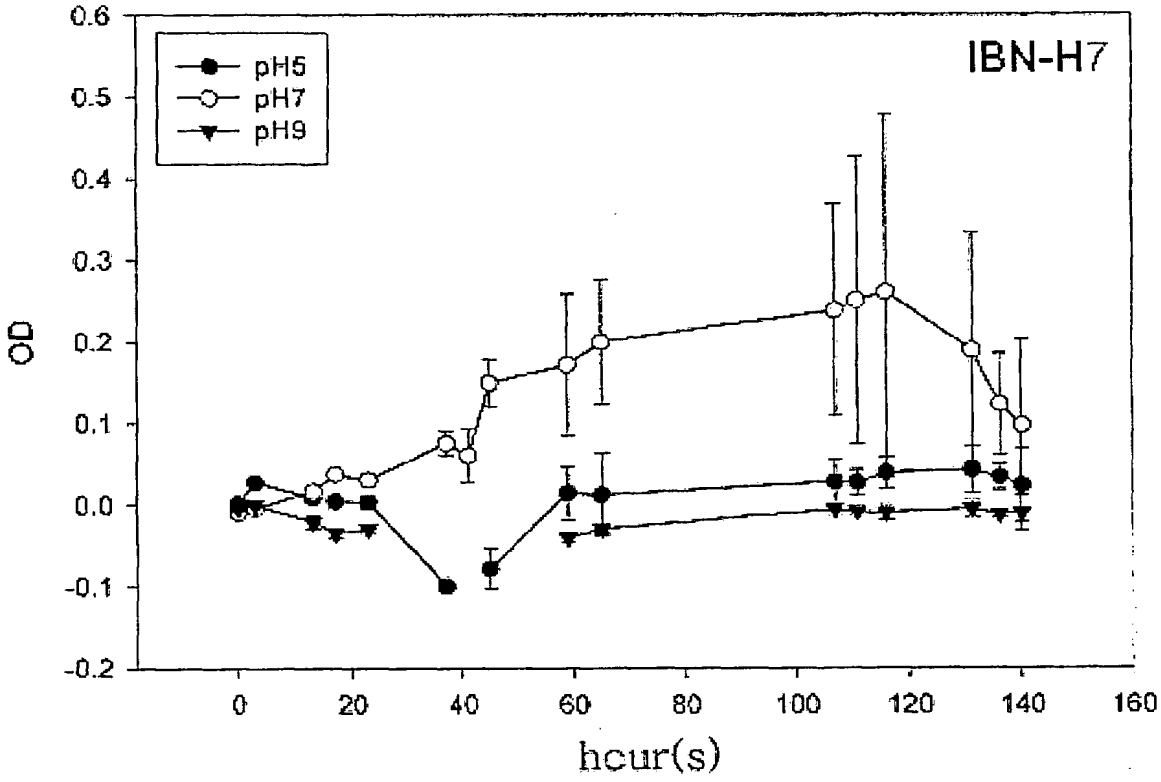
IBN-H7
pH5
pH7
pH9
OD
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 3c

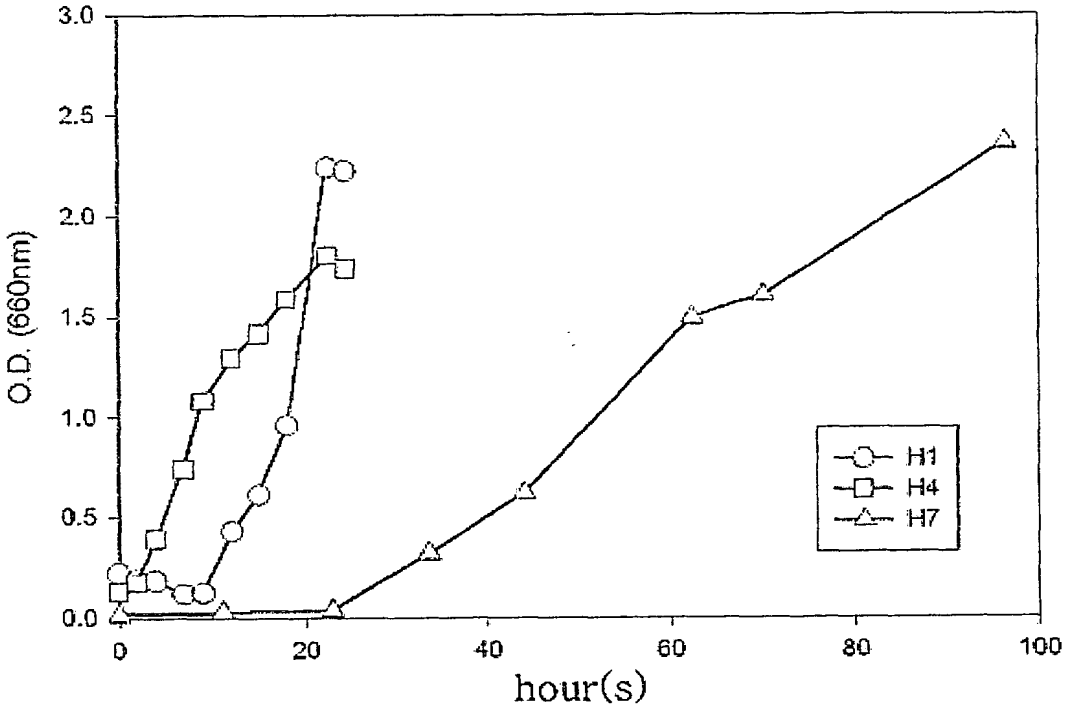

Fig. 4

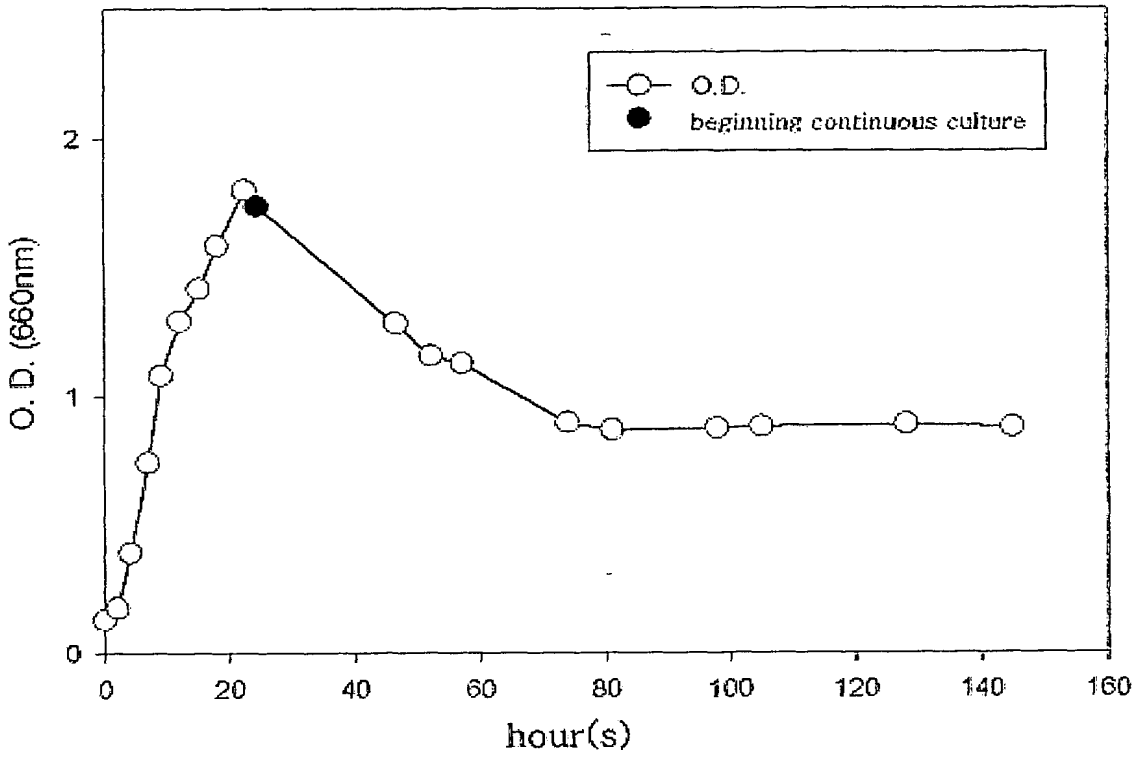
O.D.
beginning continuous culture
O. D. (660nm)
0
1
2
0
20
40
60
80
100
120
140
160
hour(s)

Fig. 5

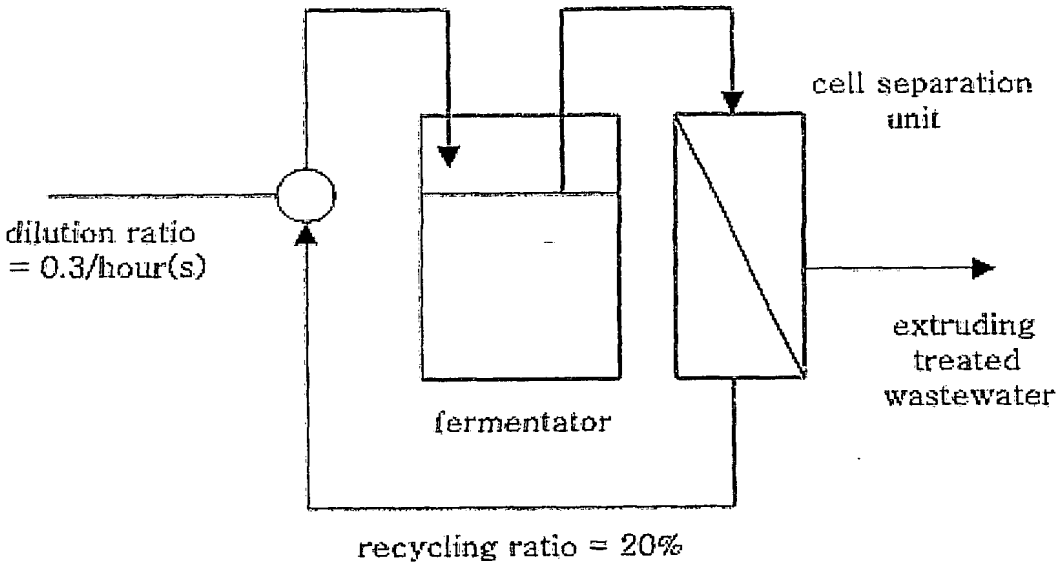
cell separation unit
dilution ratio = 0.3/hour(s)
extruding treated wastewater
fermentator
recycling ratio = 20%

Fig. 6

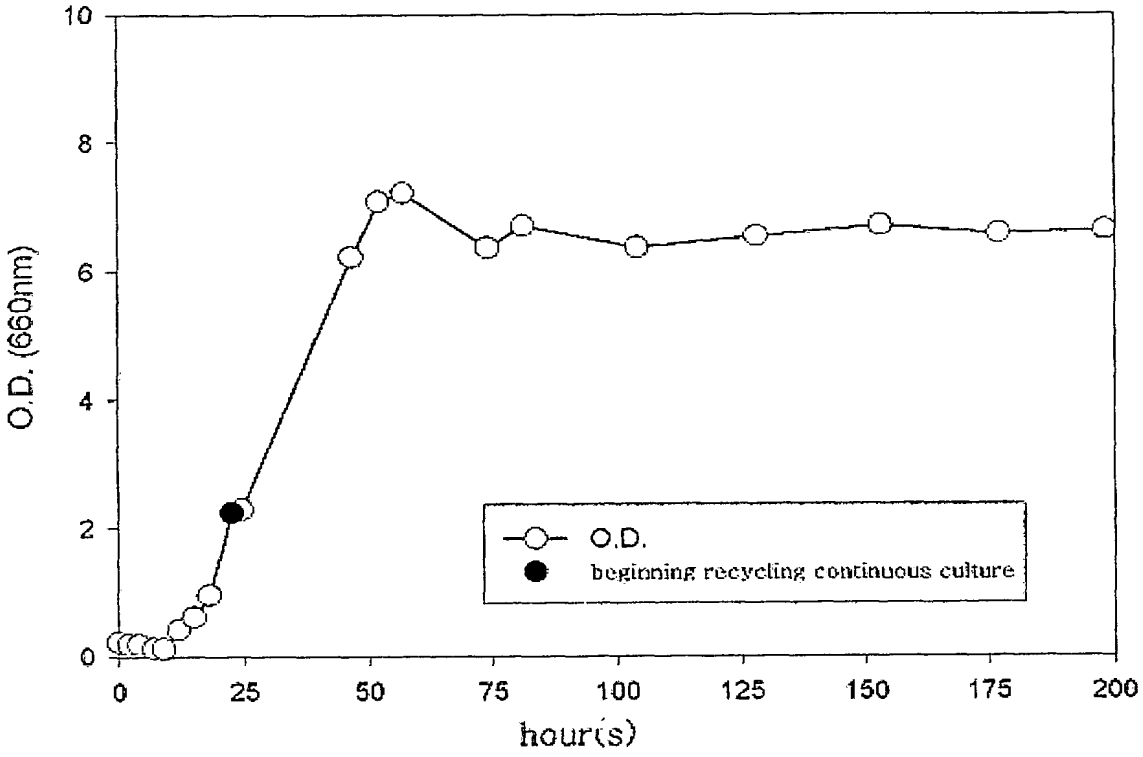
10
8
6
4
2
0
O.D. (660nm)
0
25
50
75
100
125
150
175
200
hour(s)
O.D.
beginning recycling continuous culture

Fig. 7

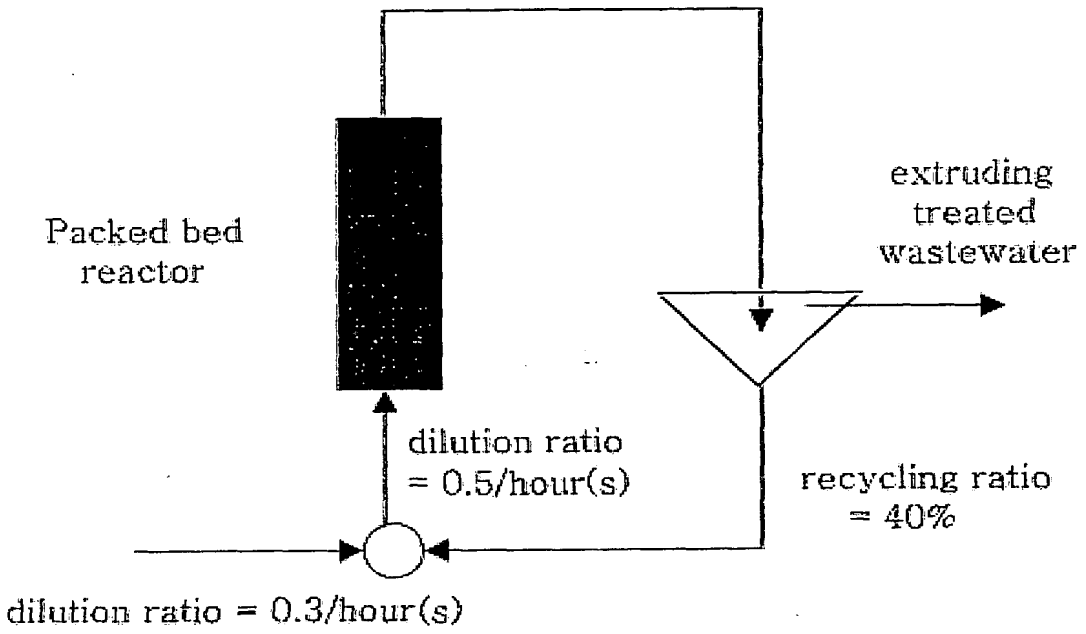
Packed bed
reactor
extruding
treated
wastewater
dilution ratio
= 0.5/hour(s)
recycling ratio
= 40%
dilution ratio = 0.3/hour(s)

Fig. 8

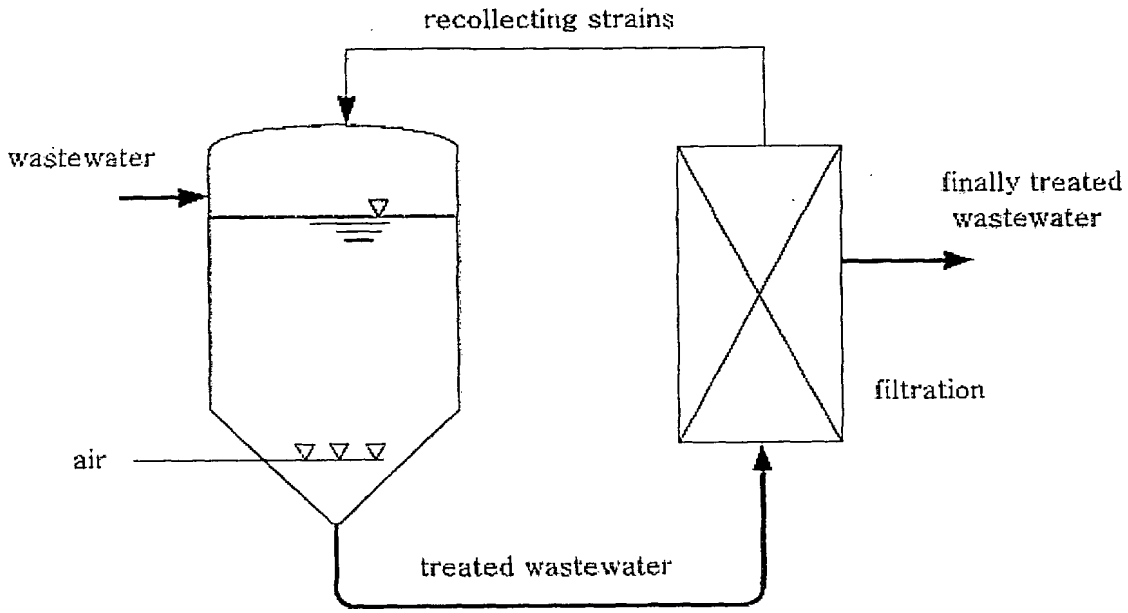
recollecting strains
wastewater
finally treated
wastewater
filtration
air
treated wastewater

Fig. 9a

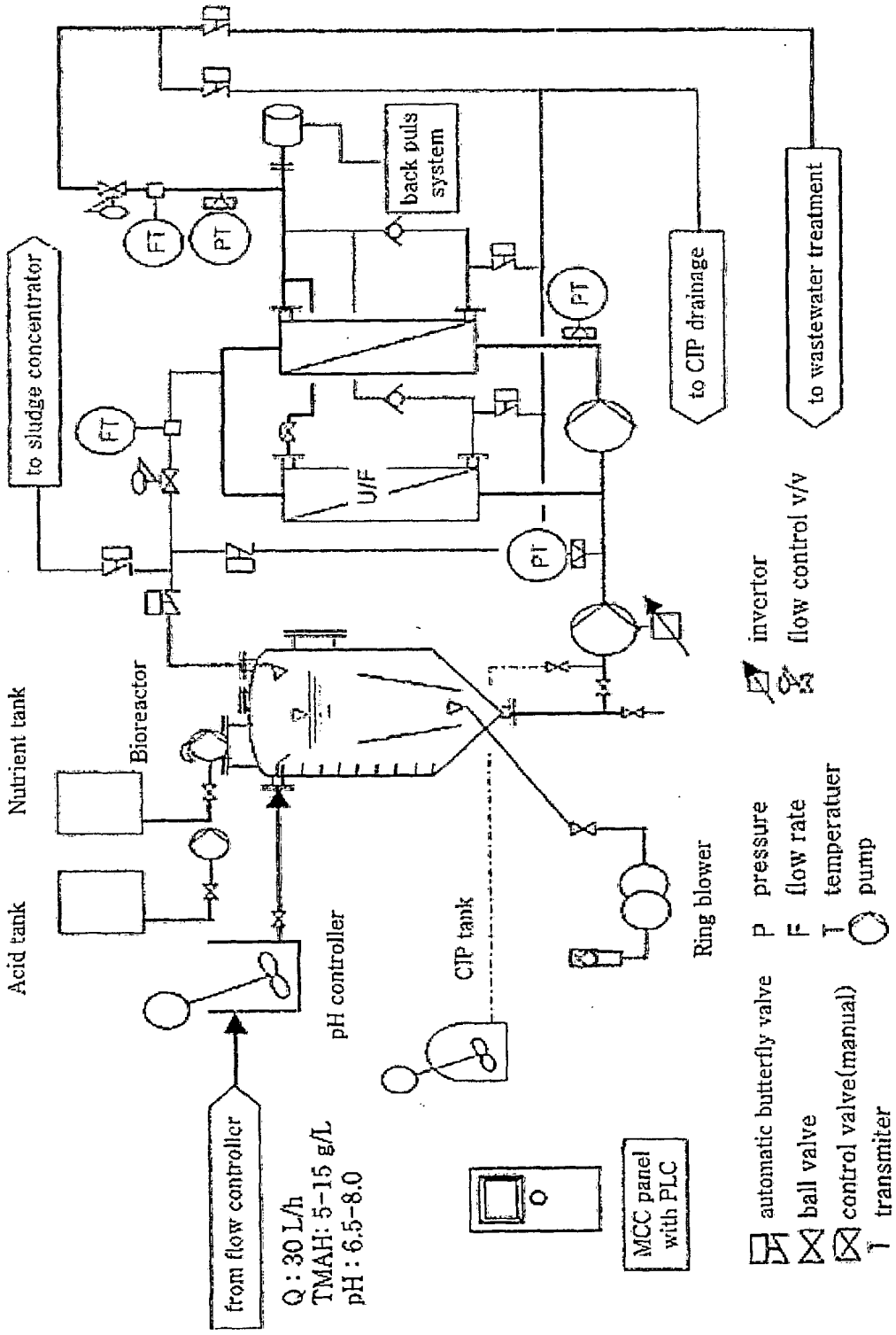
back puls system
FT
PT
to sludge concentrator
to CIP drainage
to wastewater treatment
U/F
Nutrient tank
Bioreactor
Acid tank
pH controller
CIP tank
Ring blower
from flow controller
Q : 30 L/h
TMAH: 5-15 g/L
pH : 6.5-8.0
MCC panel with PLC
inverter
flow control v/v
automatic butterfly valve
ball valve
control valve(manual)
transmiter
P pressure
F flow rate
T temperatuer
pump

Fig. 9b

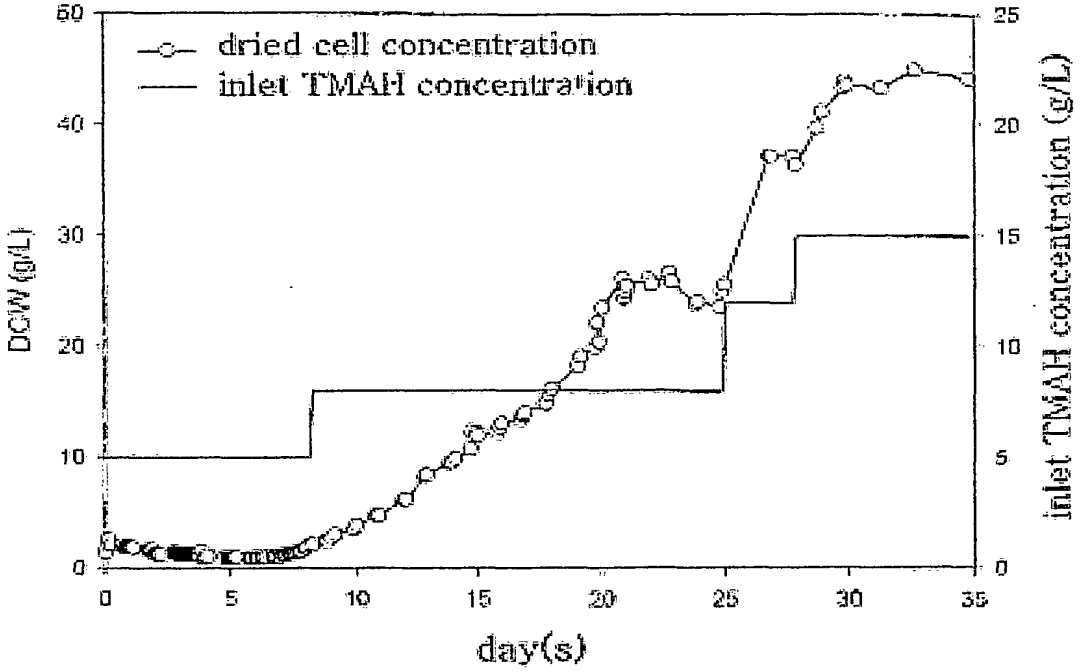
dried cell concentration
inlet TMAH concentration
DCW (g/L)
inlet TMAH concentration (g/L)
day(s)
0
10
20
30
40
50
0
5
10
15
20
25
30
35

Fig. 10

<110> INBIONET CORPORATION

<120> NOVEL STRAIN FOR DECOMPOSING TMAH, AND METHOD OF WASTEWATER TREATMENT USING THE SAME

<130> PCT01-013

<160> 2

<170> KOPATIN 1.5

<210> 1
<211> 585
<212> DNA
<213> Bacillus cereus IBN-H4

<400> 1
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tagcggcgga cgggtgagta acacgtgggt aacctgccca taagactggg ataactccgg 120

gaaaccgggg ctaataccgg ataacatttt gaacygcatg gttcgaaatt gaaaggcggc 180

ttcggctgtc acttatggat ggacccgcgt cgcattagct agttggtgag gtaacggctc 240

accaaggcaa cgatgcgtag ccgacctgag agggtgatcg gccacactgg gactgagaca 300

cggcccagac tcctacggga ggcagcagta gggaatcttc cgcaatggac gaaagtctga 360

cggagcaacg ccgcgtgagt gatgaaggct ttcgggtcgt aaaactctgt tgttagggaa 420

gaacaagtgc tagttgaata agctggcacc ttgacggtac ctaaccagaa agccacggct 480

aactacgtgc cagcagccgc ggtaatacgt aggtggcaag cgttatccgg aattattggg 540

cgtaaagcgc gcgcaggtgg tttcttaagt ctgatgtgaa agccc 585

<210> 2
<211> 517
<212> DNA
<213> Acinetobacter sp. IBN-H7

<400> 2
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gcggacgggt gagtaatgct taggaatctg cctattagtg ggggacaaca ttccgaaagg 120

gatgctaata ccgcatacgt cctacgggag aaagcagggg ctctttatga ccttgcgcta 180

atagatgagc ctaagtcgga ttagctagtt ggtggggtaa aggcctacca aggcgacgat 240

ctgtagcggg tctgagagga tgatccgcca cactgggact gagacacggc ccagactcct 300

acgggaggca gcagtgggga atattggaca atgggggaa ccctgatcca gccatgccgc 360

gtgtgtgaag aaggcctttt ggttgtaaag cactttaagt ggggaggagg cttacctggt 420

taatacctgg gctaagtgga cgttacccac agaataagca ccggctaact ctgtgccagc 480

agccgcggta atacagaggg tgcgagcgtt aatcgga 517

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/KR01/00583

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)
C02F 3/34 ; C12N 1/20

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched
Korean Patent and Application for Inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)
IPN, NPS, PAJ, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2-49576 (NEC CORP) 19 FEB 1990 see the whole document	1-7
A	US 5,532,162 (HALDOR AAMOT) 02 JUL 1996 see the whole document	1-7

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JUNE 2001 (29.06.2001)	29 JUNE 2001 (29.06.2001)
Name and mailing address of the ISA/KR Korean Intellectual Property Office Facsimile No.	Authorized officer AHN, Mi-Chung Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT01-013		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/KR01/00583	07 APRIL 2001 (07.04.2001)	22 JULY 2000 (22.07.2000)
Applicant		
INBIONET CORPORATION et al		

This International search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:
      ☐ contained in the international application in written form.
      ☒ filed together with the international application in computer readable form.
      ☐ furnished subsequently to this Authority in written form.
      ☐ furnished subsequently to this Authority in computer readable form.
      ☐ the statement that the subsequenlty furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
      ☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.
2. ☐ **Certain claims were found unsearchable** (See Box I).
3. ☐ **Unity of invention is lacking** (See Box II).
4. With regard to the **title,**
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract,**
   ☐ the text is approved as submitted by the applicant.
   ☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the **drawing** to be published with the abstract is Figure No. 8
   ☒ as suggested by the applicant.
   ☐ None of the figures.
   ☐ because the applicant failed to suggest a figure.
   ☐ because this figure better characterizes the invention.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention describes a wastewater treatment method by a microorganism decomposing Tetramethyl Ammonium Hydroxide (TMAH) which, utilized in etching the surface of silicone chip in semiconductor manufacturing process, is toxic and hard to decomposed. The present invention provides novel microorganisms capable of decomposing TMAH. Also, the present invention provides a treatment method for wastewater containing TMAH, using the microorganisms. The present invention is useful in industrial field as an environmental friendly wastewater treatment method by decomposing over 90% of TMAH, one of environmental contamination materials in the wastewater of semiconductor factory.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/00583

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

C02F 3/34 ; C12N 1/20

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Korean Patent and Application for Inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

IPN, NPS, PAJ, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2-49576 (NEC CORP) 19 FEB 1990 see the whole document	1-7
A	US 5,532,162 (HALDOR AAMOT) 02 JUL 1996 see the whole document	1-7

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JUNE 2001 (29.06.2001)	29 JUNE 2001 (29.06.2001)
Name and mailing address of the ISA/KR Korean Intellectual Property Office Facsimile No.	Authorized officer AHN, Mi-Chung Telephone No.

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

0	**For receiving Offic use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)
0-5	**Petition**	
	The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Korean Industrial Property Office (RO/KR)
0-7	**Applicant's or agent's file reference**	PCT01-013
I	**Title of invention**	NOVEL STRAIN FOR DECOMPOSING TMAH, AND METHOD OF WASTEWATER TREATMENT USING THE SAME
II	**Applicant**	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	INBIONET CORPORATION
II-5	Address:	461-6 Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
II-6	State of nationality	KR
II-7	State of residence	KR
II-8	Telephone No.	+82-42-866-9114
II-9	Facsimile No.	+82-42-866-9111
II-10	e-mail	inbionet@inbionet.com
III-1	**Applicant and/or inventor**	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	LEE, Daesang
III-1-5	Address:	202, 458-8 Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
III-1-6	State of nationality	KR
III-1-7	State of residence	KR

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

III-2	**Applicant and/or inventor**	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	LEE, Mi-Kyoung
III-2-5	Address:	306, Wunha Mansion Misu-dong 650-080 Tongyeong Republic of Korea
III-2-6	State of nationality	KR
III-2-7	State of residence	KR
III-3	**Applicant and/or inventor**	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	KANG, Key-Jung
III-3-5	Address:	415-1 Busa-dong, Jung-ku 301-030 Taejon Republic of Korea
III-3-6	State of nationality	KR
III-3-7	State of residence	KR
III-4	**Applicant and/or inventor**	
III-4-1	This person is:	applicant and inventor
III-4-2	Applicant for	US only
III-4-4	Name (LAST, First)	SHIN, Chul-Soo
III-4-5	Address:	113-1101, Samsung-Pureun Apt. Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
III-4-6	State of nationality	KR
III-4-7	State of residence	KR
III-5	**Applicant and/or inventor**	
III-5-1	This person is:	applicant and inventor
III-5-2	Applicant for	US only
III-5-4	Name (LAST, First)	YUN, Jeong-Hwan
III-5-5	Address:	105-1302, Dungji Apt. Dunsan 2-dong, Seo-ku 302-122 Taejon Republic of Korea
III-5-6	State of nationality	KR
III-5-7	State of residence	KR

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

III-6	**Applicant and/or invent r**	
III-6-1	This person is:	applicant and inventor
III-6-2	Applicant for	US only
III-6-4	Name (LAST, First)	YUM, Do-Young
III-6-5	Address:	405-1004, Expo Apt. Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
III-6-6	State of nationality	KR
III-6-7	State of residence	KR
III-7	**Applicant and/or inventor**	
III-7-1	This person is:	applicant and inventor
III-7-2	Applicant for	US only
III-7-4	Name (LAST, First)	LEE, Jung-Ki
III-7-5	Address:	407-504, Expo Apt. Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
III-7-6	State of nationality	KR
III-7-7	State of residence	KR
III-8	**Applicant and/or inventor**	
III-8-1	This person is:	applicant and inventor
III-8-2	Applicant for	US only
III-8-4	Name (LAST, First)	PARK, Kee-Don
III-8-5	Address:	109-1706, Cheonggu-Narae Apt. Jonmin-dong, Yusong-ku 305-503 Taejon Republic of Korea
III-8-6	State of nationality	KR
III-8-7	State of residence	KR
III-9	**Applicant and/or inventor**	
III-9-1	This person is:	applicant and inventor
III-9-2	Applicant for	US only
III-9-4	Name (LAST, First)	CHOI, Ho-Joon
III-9-5	Address:	103-406, Sejong Apt. Jonmin-dong, Yusong-ku 305-390 Taejon Republic of Korea
III-9-6	State of nationality	KR
III-9-7	State of residence	KR

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

III-10	**Applicant and/or inventor**	
III-10-1	This person is:	applicant and inventor
III-10-2	Applicant for	US only
III-10-4	Name (LAST, First)	KOO, Bon-Tag
III-10-5	Address:	5-401, Sindonga Apt. Yongjeon-dong, Dong-ku 300-200 Taejon Republic of Korea
III-10-6	State of nationality	KR
III-10-7	State of residence	KR
IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	KIM, Won-Joon
IV-1-2	Address:	613, Chungsa Officetel 915, Dunsan-dong, Seo-ku 302-828 Taejon Republic of Korea
IV-1-3	Telephone No.	+82-42-484-5630
IV-1-4	Facsimile No.	+82-42-482-5638
IV-1-5	e-mail	ip@timepat.com
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.	
V-6	**Exclusion(s) from precautionary designations**	NONE
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	22 July 2000 (22.07.2000)
VI-1-2	Number	10-2000-0042271
VI-1-3	Country	KR
VI-2	**Priority claim of earlier national application**	
VI-2-1	Filing date	22 July 2000 (22.07.2000)
VI-2-2	Number	10-2000-0042272
VI-2-3	Country	KR
VI-3	**Priority claim of earlier national application**	
VI-3-1	Filing date	22 July 2000 (22.07.2000)
VI-3-2	Number	10-2000-0042273
VI-3-3	Country	KR
VI-4	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1, VI-2, VI-3
VII-1	**International Searching Authority Chosen**	Korean Industrial Property Office (KIPO) (ISA/KR)

VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	6	-
VIII-2	Description (excluding sequence listing part)	25	-
VIII-3	Claims	2	-
VIII-4	Abstract	1	EZABST00.TXT
VIII-5	Drawings	15	-
VIII-6	Sequence listing part of description	2	-
VIII-7	TOTAL	51	

PCT REQUEST PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

	Accompanying Items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-15	Nucleotide and/or amino acid sequence listing in computer readable form		separate diskette
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	8b	
VIII-19	**Language of filing of the international application**	Korean	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name (LAST, First)	KIM, Won-Joon	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/KR
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

PCT (ANNEX - FEE CALCULATION SHEET)

PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	Date stamp of the receiving Office	

0-4	**Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet**				
0-4-1	Prepared using		PCT-EASY Version 2.91 (updated 01.01.2001)		
0-9	**Applicant's or agent's file reference**		PCT01-013		
2	**Applicant**		INBIONET CORPORATION, et al.		
12	**Calculation of prescribed fees**		fee amount/multiplier	total amounts (KRW)	
12-1	Transmittal fee	T	⇨	45,000	
12-2	Search fee	S	⇨	150,000	
12-3	International fee Basic fee (first 30 sheets)	b1	500,000		
12-4	Remaining sheets		21		
12-5	Additional amount	(X)	12,000		
12-6	Total additional amount	b2	252,000		
12-7	b1 + b2 =	B	752,000		
12-8	Designation fees Number of designations contained in international application		87		
12-9	Number of designation fees payable (maximum 6)		6		
12-10	Amount of designation fee	(X)	108,000		
12-11	Total designation fees	D	648,000		
12-12	PCT-EASY fee reduction	R	-154,000		
12-13	Total International fee (B+D-R)	I	⇨	1,246,000	
12-14	Fee for priority document Number of priority documents requested		1		
12-15	Fee per document	(X)	0		
12-16	Total priority document fee	P	⇨	0	
12-17	**TOTAL FEES PAYABLE (T+S+I+P)**		⇨	1,441,000	
12-19	**Mode of payment**		cash		

VALIDATION LOG AND REMARKS

13-2-1	**Validation messages Request**	Green? A translation of the international application into English will have to be prepared under the responsibility of the ISA selected.

PCT (ANNEX - FEE CALCULATION SHEET) PCT01-013

Original (for SUBMISSION) - printed on 07.04.2001 10:25:23 AM

		Green? Please note that the entire request (including the title of invention) must be in English
13-2-5	Validation messages Biology	Green? Biology: Several PCT contracting States require these indications to be included in the description. Please verify.
13-2-10	Validation messages For receiving Office/International Bureau use only	Green? Verify electronic data for consistency against printed form.

PCT

PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

0-1	**Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)
0-2	**International Application No.**	
0-3	**Applicant's or agent's file reference**	PCT01-013

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	page	15
1-2	line	9
1-3	**Identification of Deposit**	
1-3-1	Name of depositary institution	Korean Collection for Type Cultures
1-3-2	Address of depositary institution	52, Oun-dong, Yusong-Ku, Taejon 305-333, Republic of Korea
1-3-3	Date of deposit	18 July 2000 (18.07.2000)
1-3-4	Accession Number	KCTC 0834BP
1-4	**Additional Indications**	NONE
1-5	**Designated States for Which Indications are Made**	all designated States
1-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	page	15
2-2	line	10
2-3	**Identification of Deposit**	
2-3-1	Name of depositary institution	Korean Collection for Type Cultures
2-3-2	Address of depositary institution	52, Oun-dong, Yusong-Ku, Taejon 305-333, Republic of Korea
2-3-3	Date of deposit	18 July 2000 (18.07.2000)
2-3-4	Accession Number	KCTC 0835BP
2-4	**Additional Indications**	NONE
2-5	**Designated States for Which Indications are Made**	all designated States
2-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE
3	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
3-1	page	15
3-2	line	11

PCT

PCT01-013

Original (for **SUBMISSION**) - printed on 07.04.2001 10:25:23 AM

3-3	**Identification of Deposit**	
3-3-1	Name of depositary institution	Korean Collection for Type Cultures
3-3-2	Address of depositary institution	52, Oun-dong, Yusong-Ku, Taejon 305-333, Republic of Korea
3-3-3	Date of deposit	18 July 2000 (18.07.2000)
3-3-4	Accession Number	KCTC 0836BP
3-4	**Additional Indications**	NONE
3-5	**Designated States for Which Indications are Made**	all designated States
3-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	NONE

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0-4	**This form was received with the international application:** (yes or no)	
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	**This form was received by the international Bureau on:**	
0-5-1	Authorized officer	

PCT POWER OF ATTORNEY

PCT01-013

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0-1	PCT Power of Attorney (for an international application filed under the Patent Cooperation Treaty) (PCT Rule 90.4)	
0-1-1	Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)

1	The undersigned applicant(s)	INBIONET CORPORATION; LEE, Daesang; LEE, Mi-Kyoung; KANG, Key-Jung; SHIN, Chul-Soo; YUN, Jeong-Hwan; YUM, Do-Young; LEE, Jung-Ki; PARK, Kee-Don; CHOI, Ho-Joon; KOO, Bon-Tag
1-1-1	hereby appoints (appoint) the following person	KIM, Won-Joon 613, Chungsa Officetel 915, Dunsan-dong, Seo-ku 302-828 Taejon Republic of Korea
1-2	as	agent
1-3	to represent the undersigned before	all the competent International Authorities
1-4	in connection with the international application identified below:	
1-4-1	Title of the invention	NOVEL STRAIN FOR DECOMPOSING TMAH, AND METHOD OF WASTEWATER TREATMENT USING THE SAME
1-4-2	Applicant's or agent's file reference	PCT01-013
1-4-3	International application number (if already available)	
1-4-4	filed with the following Office as receiving Office	Korean Industrial Property Office (RO/KR)
1-5	and to make or receive payments on behalf of the undersigned.	

2-1	Signature of applicant	
2-1-1	Name	INBIONET CORPORATION (KOO, Bon-Tag)
2-2	Signature of applicant	
2-2-1	Name	LEE, Daesang
2-3	Signature of applicant	
2-3-1	Name	LEE, Mi-Kyoung

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2-4	Signature of applicant	
2-4-1	Name	KANG, Key-Jung
2-5	Signature of applicant	
2-5-1	Name	SHIN, Chul-Soo
2-6	Signature of applicant	
2-6-1	Name	YUN, Jeong-Hwan
2-7	Signature of applicant	
2-7-1	Name	YUM, Do-Young
2-8	Signature of applicant	
2-8-1	Name	LEE, Jung-Ki
2-9	Signature of applicant	
2-9-1	Name	PARK, Kee-Don
2-10	Signature of applicant	
2-10-1	Name	CHOI, Ho-Joon
2-11	Signature of applicant	
2-11-1	Name	KOO, Bon-Tag
3	Date	06 April 2001 (06.04.2001)


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